Investigation of the Toxic & Teratovenic Effects of GRAS Substances to the Developing Chicken Embryo-**Report of the inhouse investigations of Choline Chloride in the developing chicken embryo** 12/29/77

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MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : GRAS Review Branch, HFF-335 DATE: December 29, 1977

THRU: HFF-150

FROM : Supervisory Chemist
Whole Animal Toxicity Branch (HFF-155)

SUBJECT: Investigation of the Toxic and Taratovenic Effects of GRAS Substances to the Developing Chicken Embryo

Attached is the report of the inhouse investigations of Choline Chloride in the developing chicken embryo.

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Investigations of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo: Choline Chloride

Protocol:

Choline Chloride (1) was tested for toxic and teratovenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some to hatch.

Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of test.

Columns 1 and 2 gave the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalties observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve disorders.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton espressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

Discussion:

Choline chloride showed little toxicity under the four conditions of test. Air cell treatment at 0 hours and yolk treatment at 96 hours resulted in regression lines whose slopes were not significantly different from zero ($p = 0.05$). For the two other test conditions, the following LD_{50s} were calculated:

Air cell @ 96 hours: 221.51 mg/kg (11.08 mg/egg)
Yolk @ 0 hours: 145.93 mg/kg (7.30 mg/egg)

Scattered abnormalities were observed under all conditions of test, but serious abnormalities were in no instance significantly higher than or different from those observed in the background. Choline chloride displayed no teratogenicity under the test conditions employed.

1. Choline Chloride, Ruger Chemical Company Lot #X2502130

2. McLaughlin, J., Jr., Marliac, J. P., Verrett, N. Jacqueline, Mutchler, Mary K., and Fitzhugh, O.G., (1963) Toxicol. Appl. Pharmacol 5, 760-770

3. Verrett, N. J., Marliac, J. P., and McLaughlin, J., Jr., (1964) JAOAC 47, 1002-1006

4. Finnery, D. J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendix I.

Choline Chloride
Air Cell at 0 Hours

Table 1

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
25.00	500.00	120	37.50*	4.16	0.83
12.50	250.00	120	25.00	0.83	0.83
5.00	100.00	120	34.16	1.66	0.83
2.50	50.00	120	20.00	2.50	0.83
1.250	25.00	120	16.66	0.83	0.83
Water Controls		120	24.16	0.83	0.00
		310	7.41	2.25	2.25

*Significantly different from solvent $p \leq 0.05$
**Slope not significantly different from zero $p = 0.05$

Choline Chloride
Air Cell @ 96 Hours

Table 2

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
12.50	250.00	115	64.34*	8.69	10.43
6.250	125.00	115	32.17*	5.21	2.60
2.50	50.00	115	16.52	3.47	2.60
1.250	25.00	115	17.39	6.08	4.34
0.6250	12.50	115	17.39	1.73	0.86
Water Controls		120	15.83	5.00	4.16
		310	7.41	2.25	2.25

*Significantly different from solvent $p \leq 0.05$
**LD_{50} 221.508 mg/kg (11.075 mg/egg)

Choline Chloride
Yolk at 0 Hours

Table 3

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
25.00	500.00	120	75.83*	1.66	0.83
12.50	250.00	120	67.50*	0.00	0.00
5.00	100.00	120	61.66*	0.00	0.00
2.50	50.00	120	54.16*	0.00	0.00
1.250	25.00	120	49.16*	0.83	0.83
Water Controls		120	28.33	2.50	1.66
		310	7.41	2.25	2.25

*Significantly different from solvent $p \leq 0.05$
**Slope not significantly different from zero $p = 0.05$

Choline Chloride
Yolk at 96 Hours
Table 4

Dose mg/egg	Dose mg/kg	Number of Eggs	** Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
12.50	250.00	110	42.72*	4.54	1.81
6.250	125.00	109	45.87*	3.66	2.75
2.50	50.00	110	40.00*	2.72	0.90
1.250	25.00	110	35.45*	3.63	1.81
0.6250	12.50	109	34.86*	2.75	0.91
Water		115	17.39	0.86	0.86
Controls		310	7.41	2.25	2.25

*Significantly different from solvent $p \leq 0.05$
**Slope not significantly different from zero $p = 0.05$